Anti-aging: Review and Experimental Clinical Study of Bioavailable Calcium–Probiotics and their Effect on Reversing Osteopenia, Osteoporosis, and other Common and Chronic Health Conditions

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Received November 5, 2011; accepted November 24, 2011

ABSTRACT

This review highlights the innovative anti-aging strategies through effective use of calcium supplements. With the advent of pharmacology, modern medical technology and scientific advances in food and nutrition, the longevity of human being significantly improved. However, living long or anti-aging does not make any sense unless people maintain excellent health and quality of life. Thus, maintaining good health is the prime requirement for anti-aging. Two diseases that rob the physical fitness of the people are sarcopenia (muscle loss) and osteoporosis (loss of bone mineral density). Both of these wasting diseases can be effectively controlled with proper nutrition, lifestyle changes, and medical supplementation. This article describes the calcium supplementation and its importance in reversing and preventing osteomalacia and osteoporosis. It particularly focuses on the bioavailable calcium and the mechanism of calcium absorption in the gastrointestinal tract (GIT). The calcium transport patterns (trans-cellular and para-cellular), sites of maximum absorption of calcium in the GIT, the role of probiotics, vitamin D and dairy peptides to improve absorption of calcium have been briefly discussed. It is clearly evident that not all the calcium supplements available in the market are bioavailable. The role of calcium in controlling hypertension, cholesterol, triglycerides, obesity and reduction of colon cancer has been described. The merits of administering combination regimens of bioavailable calcium and probiotics have also been elaborated. Specific but limited clinical data has been presented demonstrating the effect of bioavailable calcium, fortified with vitamin D and probiotics with or without isoflavones, on reversing the osteopenia and controlling the osteoporosis associated symptoms. In conclusion, bioavailable calcium formulations hold promise as effective calcium supplements for better health.

KEYWORDS: Osteopenia; osteoporosis; sarcopenia; trans-cellular; para-cellular; probiotics; isoflavones.

Introduction

Aging is inevitable to any biological entity. The human being is not an exception. With the advent of modern advances in nutrition and medical sciences we are managing to live longer. Living long alone is not the answer. Long life with excellent health (physical and mental) and quality of life is the essential requisite for the ultimate happiness. We strongly believe in anti-aging. A human being can live up to 125 years and perform well as long as his/her mind is active (Reddy and Reddy, 2007). The reason for this is the marvelous nature of the central nervous system which controls our hormones, emotions, feelings, degree of alertness and ultimately to some extent the total health.

However, there are certain diseases that can deprive our looks, physical activity, mental alertness and thus prone us to early aging and death. It is advisable that no person should depend on others to carry on their daily physical activities even at an advanced age. What do we have to do to achieve such a physical fitness and what are those silent diseases which rob our physical well being and thus short cut our life span? There are two silent diseases that will deprive our physical fitness with age. They are sarcopenia and osteoporosis. Sarcopenia is the muscle loss and osteoporosis is excessive loss of calcium (bone mineral) from bones. These two conditions literally rob people’s good looks as they age. If people can conquer them, they are in the right direction to live long and lead a healthy life.

In this article, we reviewed the calcium absorption and the role of calcium in prevention of osteoporosis with a special emphasis on the bioavailable calcium formulations. It also summarized results of community-based and controlled clinical trials on the effect of bioavailable calcium-probiotics combination regimens in reversing the osteopenia and osteoporosis.
Overview of Calcium and Osteoporosis

Osteoporosis

Osteoporosis is a silent disease because bone density or mass is lost without any sign of disease or sickness. It is defined by World Health Organization (WHO) as a bone mineral density (BMD), which is 2.5 standard deviations below the peak bone mass (20 year old healthy human) as measured by DXA (dual energy x-ray absorptiometry). The above definition is particularly applied for women, since women are more prone to osteoporosis than men, although both sexes are affected with this disease (Naidu, 2009; Nordin and Morris, 2004). The following three major types of bone fractures could result from osteoporosis: (1) Vertebral crush fracture involving vertebrae in spine; (2) Colles fracture in bones of wrist and forearm; and (3) Hip fracture, which is the most serious one. In addition to the aforementioned osteoporotic fractures, the following symptoms will be evident due to severe calcium deficiency: joint problems, insomnia, nervousness, muscle spasm, irregular heartbeat, loss of hard and weak nails (especially in women), dental problems, premature aging and fatigue (Gaby, 1994). One step before osteoporosis is "osteopenia" which is 1 to 2.5 standard deviations below the peak bone mass, as expressed by T score. Nevertheless, osteopenia can be reversed irrespective of age with proper calcium supplementation and treatment.

Calcium absorption and metabolism

Calcium is a divalent cation and more than 99% is stored in the human bone and teeth. Rest of the 1% is distributed throughout the body controlling the functions of central nervous system, gastrointestinal system, heart, blood, kidneys, muscular system and hormonal system. According to WHO recommendation, the recommended daily allowance (RDA) is set at 1000 mg to 1200 mg of calcium/day. The RDA does not take into account bioavailability of calcium. A simple way of defining bioavailability is the amount of calcium absorbed and assimilated in comparison to the amount of calcium ingested. If 1000 mg calcium is consumed daily, and if all the 1000 mg is absorbed and assimilated, then such a calcium is considered 100% bioavailable. Not all calcium consumed through food or from products available in the market is bioavailable. If no calcium is supplemented or consumed through food, the level of calcium will go down in the blood. In order to cope up with the deficiency, calcium will be drawn from the bones (Marcus, 1987; Matkovic and Heaney, 1992). Thus, supplementation of bioavailable calcium is a must and it is a daily requirement. The human body cannot synthesize calcium and it must be supplemented. It is almost like an essential amino acid, i.e. human body cannot synthesize calcium from the available biochemical pathways. Thus, bioavailable calcium must be supplemented.

The absorption of calcium decreases as we age and also in women after menopause. An estimated 1 in 3 women are developing osteoporosis after menopause (Nordin et al., 2004). One possible explanation is the drop in estrogen level in women after menopause and in men it is the drop in testosterone level at andropause. In men testosterone gets converted to estrogen. According to leading research, as the level of estrogen drops, the production of hydrochloric acid drops in the stomach, which will significantly decrease the ionization of calcium (Reckner, 1985). The production of hydrochloric acid and other gastric secretions also gets reduced as we age. Calcium must be ionized in the stomach with the aid of gastric mucin and hydrochloric acid. In such ionized condition only it will be absorbed in the gastrointestinal tract. Knowing that ionization is essential to create bioavailable calcium, why don’t we take bioavailable calcium supplements to obviate any deficiencies or age? Ionized calcium must not be precipitated after it is mixed with the bile and pancreatic juice, which will raise the pH of the stomach contents to alkalinity. Thus the bioavailable calcium must be soluble, ionized and it also must not be precipitated at an alkaline pH in the duodenum.

The dietary calcium is absorbed in the GI tract at three locations: (i) duodenum and proximal part of jejunum; (ii) distal part of jejunum and ileum; and (iii) caecum and ascending colon (Naidu, 2009). Most of the ionized or bioavailable calcium will be absorbed through transcellular mechanism with the aid of enterocytes. This transcellular mechanism is an active transport system which is dependent on vitamin D, phosphorous and magnesium (Abrams and Atkinson, 2003; Bergman et al., 2009). Most of the calcium is absorbed (~ 90%) in the duodenum and proximal part of jejunum and is considered to be the most efficient route of absorption. The remaining calcium is absorbed mostly in the ileum through enterocyte cell junctions (paracellular), which is the most inefficient route of absorption (Bronner, 1996; Duflos et al., 1995; Powell et al., 1999; Bronner and Pansu, 1999; Khanal and Nemere, 2008). A small amount of calcium also gets absorbed through caecum and ascending colon (Stein, 1992; Wasserman et al., 1992; Camara-Martos and Amaro-Lopez, 2002).

Role of probiotics in calcium absorption

The healthy probiotics (beneficial bacteria) play a significant role in enhancing the calcium absorption through the paracellular route in the ileum (Reddy and Reddy, 2009). This is achieved through maintaining pH to improve the solubility of calcium. However, the predominant fraction of bioavailable calcium must be absorbed in the upper portion of the small intestinal tract. The parathyroid hormones also play a significant role in calcium absorption (Talmage and Mobley, 2007). Hormonal control aspects are beyond the scope of this article. It goes to prove that in order to eliminate bone loss or early aging, a daily supplementation of bioavailable calcium and proper probiotic is a must. In 2000, we have proved beyond doubt that the pharmacological effect of drugs is enhanced with probiotics (Reddy et al., 2000). Once the bone mass is
reduced, naturally the muscular strength and contour of the face and the body will also be altered making you look older. Thus the maintenance of bone mass is an essential requisite for health, physical appearance and anti-aging. Osteoporosis weakens mandibular bone and creates complicated dental teeth problems (Brechner and Armstrong, 1941). The bioavailability of a calcium supplement can be enhanced with the fortification of vitamin D (St-Arnaud, 2008), phosphorus (Anderson 1996; Takeda et al., 2004), magnesium (Gaby, 1994), strontium (Blake et al., 2007), zinc (Nishi, 1996; Marie 2006) and dairy related peptides and amino acids (Hansen et al., 1979; Zamel, 2003).

In addition, these are some of the well established facts regarding the relationship between calcium and osteoporosis. An inadequate intake of calcium results in low peak bone mass. This has been identified as one of the risk factors in the development of osteoporosis. In this connection, the peak bone mass is defined as the total quantity of bone present at maturity. According to the medical literature in this arena, the peak bone mass at maturity (age around 18 to 25) has the greatest bearing on whether a person will be at risk of developing either osteopenia or osteoporosis later in life, as they age. In other words, if a person has an adequate or high “peak bone mass” at adolescence (extending from about fourteen to twenty five years of age in man, and from about twelve to twenty one in woman) and early adulthood (roughly 21 to 25 years age), the chances of getting osteoporosis at a later age is considerably lower. Yet, another factor that influences the total bone mass and susceptibility to osteopenia or osteoporosis is the rate of bone loss after skeletal maturity. Even though, to some extent, the upper limit of the “peak bone mass” is genetically determined, an adequate intake of calcium is thought to exert a positive effect during adolescence and early adulthood in optimizing the amount of bone that is laid down. Ultimately all persons lose bone with age, unless adequate bioavailable calcium is consumed.

Age, race and other factors affecting calcium absorption

A person with higher bone mass at maturity or adulthood takes longer to reach the critically reduced mass at which bones can fracture easily at a later age. Conversely, the rate of bone loss after skeletal maturity at adulthood also influences the amount of bone present at old age and thus can influence an individual’s risk of developing osteopenia or osteoporosis. In order to reduce the risk of osteoporosis in the elderly men and the women (during the first decade following menopause), an adequate intake of bioavailable calcium is essential. Osteoporosis is highly prevalent in Caucasians and Asians due to their low peak bone mass at maturity. Among the Caucasians, Northern European ancestry women, experience the highest incidence of osteoporosis related bone fractures. Whereas, the American women of African heritage have the lowest incidence of osteoporosis because they have the highest peak bone mass at their adolescence (Code of Federal Regulations, 2001). People with lower specific hormonal status (Talmage and Mobley, 2007), family history, level of exercise, life style choices such as smoking and excess alcohol consumption (Spencer et al., 1985) should be extremely careful and make sure they consume adequate bioavailable calcium to minimize the risk of developing osteoporosis (Spencer et al., 1982; Hernandez-Aviala et al., 1991). In many Asians, the consumption of milk and dairy products are significantly lower due to majority of the population has lactose intolerance and allergy to intact milk proteins (Reddy and Reddy, 2007). Consequently, they are more susceptible to develop osteoporosis. This can be prevented through supplementation of bioavailable calcium, enzyme hydrolyzed lactose and proteolyzed milk proteins. Nevertheless, it is advisable to take bioavailable calcium supplement throughout the life to minimize or override this silent disease i.e. osteoporosis.

Calcium supplementation in osteoporosis and other conditions

In addition to reducing the risk of osteoporosis, calcium has also been credited with reducing hypertension, cholesterol, triglycerides, obesity and the incidence of colon cancer (McCaroon and Morris 1985; Resnick et al., 1983; Slattery et al., 1988; Wargovich and Baer, 1989; Bierenbaum et al., 1972; Zamel, 2003). In experimentally controlled clinical trials, it has been proven that patients who received 1000 mg of calcium per day for eight weeks have shown a decrease of an average of 3.8 to 5.6 mmHg for systolic and 2.3 mmHg for diastolic blood pressure (Resnick et al., 1983; and McCaroon and Morris, 1985). In our own experience, we have observed people who took bioavailable calcium on a daily basis did not have any signs of elevated blood pressure for over ten years and longer, even though they had a family history of hypertension. Also, it has been proven through clinical research that taking calcium supplement can control hypertension, which is a leading cause of heart diseases.

In a separate study by Bierenbaum et al. (1972), it was evident that calcium supplementation had a significant effect of lowering cholesterol (a supplementation of 800 mg of calcium per day reduced serum cholesterol from 349 mg/dl to 278 mg/dl in 6 months) and triglycerides (the average serum triglyceride level fell from 327 mg/dl to 213 mg/dl in one year).

Dental clinical research by Krook et al. (1972) proved that a supplementation of 1000 mg of calcium daily for six months significantly reduced the bleeding of gums, tooth mobility and completely eliminated gingivitis. Also, new alveolar bone appeared in some cases, signifying daily calcium supplementation is the best way to reduce the periodontal diseases. Elimination of loss of alveolar bone is a sure way to maintain a good facial contour and thus good looks, in addition to the health. Dentists can take advantage of these findings and recommend daily supplementation of bioavailable
calcium (a minimum of 1000 mg/day) for their patients after oral surgery, to speed up the recovery and build the stronger alveolar bone.

The published evidence clearly indicate that adequate ingestion of calcium decreases the incidence of colon cancer through reduction of proliferation of colonic cells (Slattery et al., 1988; Wargovich and Baer, 1989). This effect was more pronounced in men than women. In other words, more bioavailable calcium in diet lowers the risk of colon cancer. Although more research is required in this area, the indications are that calcium has more beneficial effects, in addition to reducing the risk of osteoporosis, in both men and women.

**Calcium and reduction of obesity**

Extensive research on the role of calcium in reducing adiposity by Zemel (2003) proved that dairy origin calcium exerts substantially greater effect on both fat loss and fat distribution compared to an equal amount of other non-dairy source supplemental calcium (such as shell calcium, calcium carbonate and calcium citrate). Dietary calcium plays a vital role in the regulation of energy metabolism. The high calcium diets lower both the adipocyte lipid accumulation and weight gain during periods of over consumption of high energy or high calorie diets and at the same time increase lipolysis (fat breakdown) and preserve thermogenesis during caloric restriction diet periods, thereby markedly speeding up weight loss. In other words, high calcium intake helps people to lose weight both during dieting and also during regular non dieting periods. It has been demonstrated that low calcium diets stimulate the production of calcitrol, which in turn stimulates calcium influx in human adipocytes and thereby promotes adiposity. Taking this into account, the calcitrol production can be lowered by administering more calcium, which will in turn reduce adiposity or obesity. This is an attractive way and relatively easy way to make a person lose weight. The dairy source calcium has significantly greater effect in decreasing the weight and fat gain and increasing the fat loss. The possible reason for the superior effect of dairy calcium is probably due to additional non-specific bioactive compounds (peptides) in dairy which act synergistically with calcium to lower the adiposity. Angiotensin converting enzyme inhibitory peptides which are of dairy origin may reduce the adiposity. These concepts are confirmed and supported by epidemiological and clinical data. Considering the above research findings by Zemel (2003), dairy origin bioavailable calcium should significantly reduce obesity, which is one of the most dangerous diseases affecting the majority of population in the Western world.

**Probiotics facilitates calcium absorption**

An interesting question can be raised at this juncture. If the milk can supply all the dairy source calcium, why does North European descent Caucasian population, who consume lot of milk in their daily ration, get effected with calcium deficiency diseases such as osteopenia and osteoporosis? To answer this question, we propose the following hypothesis. The absorption of calcium in the GI tract, according to current understanding, greatly depends on some non-specific peptide fractions derived from milk. There are two types of major proteins in the milk. They are casein (2.5%) and whey proteins (0.7%). These two proteins have to be proteolyzed to release the smaller peptides. Since most of these peptides are released in the lower intestinal tract, the availability of such peptides is scant in the jejunum region. In some individuals, if the digestive efficiency is lower, the peptide formation is delayed, which will indirectly reduce the calcium absorption (Epstein et al., 1982). It makes sense because, especially in woman (after menopause) and older men, the digestive efficiency goes down due to lower production of hydrochloric acid, mucin and other gastric enzymes (Naidu, 2009). To override this factor, it is advisable to fortify the bioavailable calcium with dairy peptides. Since the digestive efficiency of no two individuals is same, and also it depends heavily on age, sex and life style, it is advisable to take proper bioavailable calcium-peptide supplements fortified with probiotics to override such unavailable factors which hamper calcium absorption.

In this connection we would like to elaborate briefly about probiotics, which are involved in improving the calcium absorption in the distal end of the small intestinal tracts. What are probiotics and how do they improve human health? Probiotics are bacteria or microorganisms that are beneficial to the health of an individual. As early as 1907, the Nobel Laureate Dr. Metchnikoff discovered that lactic acid producing bacteria administered orally stopped intestinal ailments in humans (Reddy and Reddy, 2009). It has been well established clinically that the intestinal ailments significantly reduce the calcium absorption (Molteni et al., 1990). Probiotics are essentially the opposite of antibiotics, which are inhibitory to other bacteria (including probiotic bacteria). The word “probiotic” is derived from two Greek words, “Pro” and “Bios”, meaning “for life.” Fuller (1989) redefined probiotics as live microbial supplements that bestow beneficial effects on the host by improving the intestinal microbial balance.

Previously we suggested that modern foods which contain preservatives and even the physician prescribed antibiotics drugs are not beneficial to the gastrointestinal flora, especially probiotics (Reddy and Reddy, 2007; 2009). If all or most of the probiotics are destroyed in the GI tract, the human life is in jeopardy because of eco-imbalance of the GI tract flora (Bengmark, 1998). Consequently, in order to maintain and ensure proper GI eco-flora, continuous or periodic supplementation of probiotics is a must.

**Benefits of probiotic supplements**

The following are some of the benefits of probiotics to improve health:
1. **Reduction of lactose malabsorption:** Half of the world population is unable to utilize lactose (milk sugar) (Reddy and Reddy, 2004; 2005; 2007; 2008; 2009). Lactose malabsorption can be corrected by probiotics (Fuller, 1989). Lactose is a disaccharide made of glucose and galactose. A person who lacks the enzymes lactase in the gut cannot digest lactose and thus develops clinical symptoms of typical lactose intolerance such as flatulence and diarrhea. Probiotics, especially *L. acidophilus*, have the β-galactosidase enzyme, which can break down the disaccharide lactose into the simple sugars glucose and galactose in the ileum, and thus reduce the discomfort of lactose intolerance. Recently more people are exhibiting fructose intolerant and proper probiotics may greatly improve such discomfort.

2. **Reduction of intestinal infections:** Antibiotic-induced diarrhea has been successfully controlled by probiotics. Specific strains of *Lactobacillus* and *Bifidobacterium* have been used in children and adults for therapy of intestinal infections (Reddy and Reddy, 2009). The mechanism of action of probiotic to reduce intestinal infections is through competition for gut nutrients, secretion of anti-microbial substances, reduction of pH through short chain fatty acid formation, blockage of adhesion sites for pathogens, blockage of toxin receptor sites, immune stimulation and attenuation of virulence of unwanted bacteria and virus. (Reddy and Reddy, 2004).

3. **Reduction of coronary heart disease:** The fact that probiotic supplementation decreases cholesterol concentrations in the blood and increases the excretion of cholesterol in the feces has been well documented (Bierenbaum et al., 1972; Agerback et al., 1995). Cholesterol absorption is interfered within the gut partly due to assimilation of cholesterol by probiotic organisms (Gilliland et al., 1985). In addition, probiotics (*Bifidobacterium longum*) deconjugate bile salts with aid of bile salt hydrolase, and thus increase the excretion of free bile salts in the feces. This has the potential to reduce serum cholesterol because the replacement of bile salts would require the utilization of some cholesterol in the body. Thus, the resultant hypocholesterolemia may reduce the incidence of coronary heart disease. In addition, probiotics improve the absorption of calcium form GI tract by lowering or maintaining the proper pH (Reddy and Reddy, 2009). Calcium deficiency is one of the prime factors for hypertension (McCurnon and Morris, 1985; Resnick et al., 1983). In addition, probiotics also inhibit microorganisms which convert non-toxic amino acids to toxic amines (Reddy and Reddy, 2007). Thus, the reduction of hypertension inducing amines (histamine and tyramine) in GI tract due to probiotics can also be a factor for the reduction of coronary heart diseases.

4. **Immunomodulation:** This is a very important activity induced by probiotics. Immune-stimulating activity is attributed to their bacterial cell envelope constituents such as peptidoglycan. Results of various investigators indicate that probiotics stimulate the production of antibodies, enhance the systemic activity of macrophages, and increase interferon levels and the number of killer cells, thus improving the immunity and also reduce allergies. In order to influence the immune system, probiotics have to activate the lymphoid cells of the gut associated lymphoid tissue, which are diffusely distributed among the epithelial cells and populate the lamina propria and submucos (Madera, 1997). The immunogenic properties of probiotic bacteria such as propionic acid bacterium did not reside in extracellular slime (Reddy et al., 1973). Apparently, the cell walls of these bacteria have antigenic properties, rather than their extracellular slime, further proving the immune modulating effect of probiotics is a function of their cell wall composition and thus can vary among probiotics.

5. **Suppression of cancer:** Dietary intake of lyophilized cultures of *Bifidobacterium longum* has significantly suppressed the development of azoxymethane-induced aberrant crypt foci (ACF) formation in the colon (Reddy, 1998). McIntosh (1966) outlined the ability of the probiotic strains to inhibit the incidence of colon tumors. Some of the non-probiotic type of GI flora will convert procarcinogens to carcinogens, which are the causative agents for cancer. Some of the end products of digestion are procarcinogenic. For example, end products of the digestion of beef may be more procarcinogenic than those of vegetables. Procarcinogenic materials may not cause cancer; however, if they are acted upon by certain microbial enzymes (β-glycosidase, β-glucuronidase, steroid-7α-dehydroxylase, nitroreductase and nitrate reductase, azoreductase, and tryptophanase) in the GI tract, they will be converted to carcinogens (Rowland et al., 1985; Goldin, 1986; Nagengast et al., 1995; Eberhardt, 2003). Probiotics, by their innate nature, suppress the growth of these undesirable enzyme-producing non-probiotic bacteria, and thus reduce the chance of converting procarcinogens to carcinogens (Reddy and Reddy, 2005; 2007; 2009).

6. **Suppression of Helicobacter pylori, Methicillin resistant Staphylococcus aureus (MRSA-Superbug) and Clostridium difficile (C. diff):** *Helicobacter pylori* is a Gram-negative spiral-shaped bacterial pathogen that colonizes in the area between the mucous layers of stomach and gastric epithelium. It is now recognized as the causative agent for chronic gastritis, which is a major factor for causing gastric and duodenal ulcers, gastric atrophy and gastric cancer. The International Agency for Research on Cancer has classified *H. pylori* as a Group I carcinogen (McFarlan and Munro, 1997). This is the first pathogenic bacterium to be classified as such. The methicillin resistant *Staphylococcus aureus* (MRSA) and antibiotic resistant *Clostridium difficile* (C. diff) are categorized as lethal hospital associated infections killing over 30,000 people (per annum) in
the U.S. alone. Limited research data indicate that even H. pylori, MRSA, and C. diff can be inactivated or suppressed with the use of proper probiotics (Reddy and Reddy, 2008).

As described earlier, probiotics lower the pH in the ileum through the production of lactic acid, which is very conducive for the transport and/or absorption of calcium in the distal part of the small intestinal tract. It is getting more and clearer that unless proper probiotics are ingested and maintained in the ileum, calcium absorption will be hampered. With proper probiotics, even if the active transport of calcium is hindered in some individuals due to pathological conditions, in the jejunum region, the passive transport in the ileum and caecum region can be enhanced significantly. In addition, probiotics will significantly improve the absorption of calcium in the gastro intestinal tract by reducing the intestinal infections, food intolerances, and allergies.

Experimental Clinical Study of Bioavailable Calcium Formulations

Bioavailable calcium formulations have been prepared and evaluated in patients in two distinct clinical studies: (i) community-based clinical trial; and (ii) controlled experimental clinical trials. The general formulation aspects of bioavailable calcium products and findings from these clinical trials are briefly described below.

Pharmaceutical development of bioavailable calcium products

Bioavailable dairy calcium was purchased as raw material from International Media and Cultures (IMAC Inc., Denver, Colorado, USA). The bioavailable dairy calcium had the following chemical and microbiological specification, which are outlined in Table 1 and Table 2.

TABLE 1
Composition of IMAC’s bioavailable dairy calcium per gram.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Quantity per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>260 mg to 300 mg (average 280 mg)</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>140 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>0.0437 mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>3.22 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>2.80 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.02 mg</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>&lt; 0.02 mg</td>
</tr>
<tr>
<td>Fat</td>
<td>&lt; 0.04 g</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>&lt; 0.04 g</td>
</tr>
<tr>
<td>Protein</td>
<td>0.020 g</td>
</tr>
<tr>
<td>Ash</td>
<td>0.80 g</td>
</tr>
<tr>
<td>Moisture</td>
<td>73 mg</td>
</tr>
</tbody>
</table>

Chemical tests were conducted using the test procedures outlined in the AOAC methods - 16th edition.

TABLE 2
Microbiological specifications of IMAC’s bioavailable dairy calcium per gram.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform</td>
<td>&lt; 10 CFU/gram</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>&lt; 10,000 CFU/gram</td>
</tr>
<tr>
<td>Yeast</td>
<td>&lt; 100 CFU/gram</td>
</tr>
<tr>
<td>Meld</td>
<td>&lt; 100 CFU/gram</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Negative</td>
</tr>
<tr>
<td>Listeria</td>
<td>Negative</td>
</tr>
<tr>
<td>Coaculase pos. staph</td>
<td>Negative</td>
</tr>
<tr>
<td>E. Coli</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Microbiological testing procedures were performed according to the test procedures outlined in the standard methods for the examination of dairy products and FDA bacterial analytical manual.

The following freeze dried probiotics were included in the study: *Lactobacillus acidophilus* DRK; *Bifidobacterium bifidus* MS; and *Lactobacillus sporogenus*. The microbial and spore concentrations were adjusted to arrive at 1 billion organisms or spores per gram of the freeze dried culture. The probiotic preparations were plated and assured for the absence of pathogens, according to the procedures of FDA bacterial analytical manual.

Two different calcium tablets were prepared as dietary supplements and labeled using the proprietary names of ADFAC Laboratories Pvt. Ltd. (Medipally Village, Uppal, Hyderabad, India). They are DairiCal and DairiCal Plus. The compositions of these two preparations are outlined in Table 3.

TABLE 3
The analytical composition and probiotic concentration of the DairiCal and DairiCal Plus 1000 mg tablets.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity in DairiCal</th>
<th>Quantity in DairiCal Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>225 mg</td>
<td>225 mg</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>112 mg</td>
<td>112 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>42 mg</td>
<td>42 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>8 mg</td>
<td>8 mg</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>300 IU</td>
<td>300 IU</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>1 million CFU/tablet</td>
<td>1 million CFU/tablet</td>
</tr>
<tr>
<td>B. bifidus</td>
<td>1 million CFU/tablet</td>
<td>1 million CFU/tablet</td>
</tr>
<tr>
<td>L. Sporogenes</td>
<td>1 million CFU/tablet</td>
<td>1 million CFU/tablet</td>
</tr>
<tr>
<td>Iron</td>
<td>0.035 mg</td>
<td>0.035 mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>2.576 mg</td>
<td>2.576 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.016 mg</td>
<td>0.016 mg</td>
</tr>
<tr>
<td>Soy isoflavones</td>
<td>0</td>
<td>30 mg</td>
</tr>
</tbody>
</table>
Other inert ingredients included in the tablets are microcrystalline cellulose, sodium starch glycolate, guar gum, t alc, and magnesium stearate.

Clinical study #1: Community-based clinical trial (as a complementary and alternative therapy)

(a) Materials, study protocol, and subjects. The above nutritional supplement preparations (tablets) were distributed to 100 physicians to study their effect on assisting in reducing the symptoms of osteopenia and osteoporosis in their patients. This study was conducted in India. Each physician was asked to pick 20 patients and randomly segregate them into four groups (5 in each group) on the basis of symptoms of mild osteopenia, moderate osteopenia, mild osteoporosis and severe osteoporosis. The physicians were also asked to segregate the patients not only on severity of symptoms but also on the basis of age group i.e. below forty years and above forty years. They were asked to give 900 mg calcium per day for mild osteopenia patients. Whereas, 1350 mg calcium per day for severe osteopenia patients. They were asked to give DairiCal Plus with isoflavone for the patients who are 40 years and above only. For the mild osteoporosis patients a calcium dose of 1800 mg/day, and 2250 mg/day calcium for the severe osteoporosis patients was the suggested dosage. Also, calcium supplement with isoflavones (DairiCal Plus) was to be given only to the patients who are over 40 year’s age. Since it was a randomized prevention clinical trial, male and female segregation was not done in the groups.

The physicians were asked to study their patients for a period of six months and report the clinical data. They were asked to pay particular attention to the improvement of symptoms and for any adverse side effects. They were also asked to keep track of number of patients dropped off the test, if it is significant. Since this study involved close to 2000 patients, it can be construed as phase III clinical trial, although it is not conducted as a controlled trial.

(b) Results and discussion of clinical findings. According to the physicians participated in the community-based clinical trials, the results were extremely satisfactory. Most of the osteopenia patients reported full recovery on the basis of symptoms and their physical condition. Apparently isoflavone addition was not required if the subjects age is below forty years. The subjects who are over forty with moderate osteopenia have reported excellent recovery with the use of DairiCal Plus. Most of the mild osteopenia patients showed excellent recovery even after taking bioavailable calcium (900 mg/day) for a period of two months. Whereas advanced osteopenia patients who were taking 1350 mg calcium per day showed excellent progress around 3.5 to 4 months period.

In the case of osteoporosis patients, the mild osteoporosis patients recovered very well taking a dose of 1800 mg/calcium per day for a period of four months. Whereas, in the case of advanced osteoporosis patients the treatment has to be continued at a daily dose of 2250 mg calcium/day for a period of six months. Approximately 60 to 75% of the patients reported recovery from osteoporosis on the basis of symptoms. Isoflavone addition to the bioavailable calcium seems to improve the condition of the patients who are over 40 years. According to the physicians, close to 20% of the patients did not turn up to report their condition. Since these are community-based clinical trials ran in India, it was not possible to obtain the T scores using the dual energy x-ray absorptiometry. According to the physicians, some patients went and got bone density measurements done and they have reported significant improvement. None of the patients participated in this community-based clinical trial reported any significant adverse reactions. The advanced osteoporosis patients who were asked to take bioavailable calcium at a dose of 2250 mg/day reported slight diarrhea in the beginning of the trial. However, it subsided a few days later. The recovery was judged on the basis of symptoms i.e. least or no pain in the joints, muscles, and excellent sleep pattern with least pain in the middle of the night and least pain during walking.

Most of the patients reported overall improvement in their health. Most of the women patients reported good hair growth, nails and excellent improvement in the texture and complexion of their skin. Although men did not pay much attention, reports indicated that there hair growth and skin condition and complexion also improved significantly. This massive community-based clinical trial distinctly proved that bioavailable calcium has a significant effect in reversing the osteopenia and osteoporosis, in addition to improving their overall health and appearance. We could not compare the non-dairy base calcium Vs bioavailable dairy calcium in these clinical trials. However, according to the physicians no such dramatic and significant improvement was observed with other non-dairy source calcium supplementation in their other patients outside of this clinical trial.

Clinical study #2: Controlled experimental clinical trial (as a complementary and alternative therapy)

(a) Materials, study protocol and subjects. This study was conducted in the United States. Two female candidates were recruited for this study. Both of them were Northern European ancestry Caucasian American women. The first women with confirmed osteoporosis (T score-minus 3.32) was 58 years old and she had a hysterectomy at the age of 25. She had developed osteoporosis apparently due to reduction of estrogen hormone. Although she was medically treated, with least success, to reverse osteoporosis, she had constant complaints about joint pains, muscle cramps, sleeplessness, and unexplainable pain in the bones.
The second women was 65 years old (also Northern European ancestry Caucasian American women) with confirmed osteopenia (T score minus 1.37). She also reported weakness, joint pains, and unexplainable pain in the bones. Both candidates were given 2000 mg/day bioavailable calcium supplement with no isoflavones for a period of 6 months. They both were tested for the bone density using dual energy x-ray absorptiometry (DXA) after 4 months and 6 months supplementation. Although this study is very limited, it was done under very strict conditions and supervision by monitoring their diet, exercise, and life style patterns. It is very hard in the United States to obtain well established and defined osteopenia and osteoporosis patients who are willing to participate and follow the protocols, including their diet, exercise, and complied in reporting the symptoms. The subjects recruited for this study are extremely reliable and honest.

(b) Results and discussion of clinical findings. Excellent results were obtained with the controlled experimental clinical trials ran under strict conditions in the United States. The 58 year old, Northern European ancestry Caucasian American woman, who had hysterectomy at the age of 25, reported great improvement in terms of the symptoms, i.e., Least pain in the joints, least or no muscle cramps, improved sleeping pattern, least tiredness and no pain in the bones. However, her T score only improved from −3.32 to −3.07. According to her physician she never had such an improvement before, and taking 2000 mg of bioavailable calcium/day for a period of six months greatly improved her over all physical condition. According to the patient, she never felt this good when she took non dairy base calcium supplements available in the market. Although she was not cured of osteoporosis, she was feeling much better than before in terms of reduced or no pain in the joints, no muscle cramps etc. in addition, she has witnessed luxurious hair growth, improved complexion, good growth of nails and excellent sleep pattern. The possible reason the bioavailable calcium could not cure the osteoporosis completely in this case (although it improved) because of the history of the subject, i.e., early hysterectomy at the age of 25. Apparently her peak bone mass was low at her adolescence. If we have administered DairiCal Plus (with isoflavone), perhaps this subject must have recovered better from osteoporosis, as we have observed with the patients in the community based clinical trial.

In another subject, who is 65 years old Northern European ancestry Caucasian American woman with established osteopenia, oral administration of 2000 mg of bioavailable dairy base calcium completely cured osteopenia in the shortest time. The bone density test prior to taking bioavailable calcium was as follows: Z score 1.28, T score minus 1.37. After four months span (of taking 2000 mg calcium/day) the following test results were obtained: Z score plus 2.70, and T score 0.98. According to the test results, the patient had osteopenia and after taking bioavailable calcium along with vitamin D3 and probiotics (L. acidophilus, B. bifidus, and L. sporogenes) she was completely cured. Unlike the other subject, she did not have hysterectomy and perhaps had a better peak bone mass at her adolescence. Although, these are limited studies, they were done under the strict controlled conditions. Tests were conducted under the supervision of physicians. Surprisingly, the second subject (who is 65 year old) also reported that she had a good growth of hair, better skin complexion and felt very good in terms of general condition, after taking the bioavailable dairy calcium for 4 months.

In all most all the cases, that is, community-based clinical trials and controlled experimental clinical trials, none of the patients reported any significant adverse side effects. The most interesting observations which came out of these clinical trials, in addition to improving or curing osteopenia and osteoporosis are “excellent hair growth, good skin complexion and texture, and luxurious strong nails”. As we have indicated earlier, irrespective of the health condition and age, daily ingestion of bioavailable dairy base calcium supplement fortified with vitamin D3 and proper probiotics is the best way to minimize bone loss and thus to improve physical looks and anti-aging process.

Conclusions

Overall, more than thousand patients (male and female) with osteoporosis and osteopenia showed improvement of their condition upon taking bioavailable calcium for a period of six months. Most of these patients are continuing their supplementation. The most intriguing observation in the female sector besides significant reduction of osteopenia and osteoporosis is the luxurious growth of their hair, and stronger nails, and significant improvement of skin complexion, which are all excellent signs of anti-aging. Since most of these patients are out-patients, physicians could not monitor their food habits, exercise patterns, and other finer details. Most of the mild osteopenia patients were asked to take 1000 mg of bioavailable calcium/day. Osteoporosis patients were asked to take 2000 mg bioavailable calcium/day. Bioavailable calcium preparations were enriched with probiotics and vitamin D3 when administered to patients who are under 40 years. In the case of advanced osteoporosis, patients were administered with bioavailable calcium, probiotics, Soy isoflavone and vitamin D. The results distinctly proved that for younger population, bioavailable calcium, probiotic, vitamin D was sufficient. In older population, the addition of Soy isoflavone (to the above formula) was highly beneficial.

Not all the calcium supplements available on the market are bioavailable (Nicar and Pak, 1985; Miller, 1988; Nilsen et al., 1978). Soluble calcium salt such as calcium citrate is not construed as the bioavailable source of calcium. Thus, a supplement consisting of a mixture of soluble dairy source bioavailable calcium salts, essential minerals such as phosphorus and magnesium, non- specific dairy related peptides and amino acids, along with proper and selective probiotics is
an ideal approach to assure the absorption of calcium. It is proven that several diseases, and the medications used to cure such diseases, may also have an adverse affect on the calcium absorption (Avioli, 1990; Hunt and Johnson, 1983; Spencer and Kramer, 1983). Such conditions are food allergies and food intolerances (Molteni et al., 1990; Mora et al., 1993). It has been proven that probiotics reduce the intestinal infections, allergies and food intolerances (Reddy and Reddy, 2009). Thus, taking bioavailable calcium and proper probiotic supplement, on a daily basis, is the best way to assure the health and anti-aging. Even the younger population should take supplements to ensure proper bone formation to over ride the future problems. Every individual should plan way ahead of time while they are young regarding the kind of health (physical and mental) and good physically attractive looks they would like to attain and maintain to extend their life (age) and to lead an excellent quality of life. On the contrary with the modern advances in nutrition, understanding of the psychological concepts and all natural treatment modalities, health can be built irrespective of age. However, it is advisable to start the program at an early age. Good health is inevitable to have a long healthy life, which can also be termed in simple words as anti-aging.

Acknowledgements

We are extremely thankful to all the hundreds of physicians and thousands of patients who have participated in the clinical trials both in India and in the United States of America. Sincere thanks and appreciation go to all the staff of International Media and Cultures (IMAC – USA) and ADFAC Labs, Pvt. Ltd – India, for manufacturing bioavailable calcium, preparing and distributing the DairiCal and DairiCal Plus tablets on a timely basis for this elaborate clinical study.

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